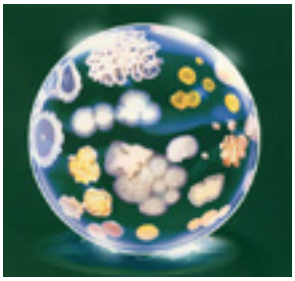

Todar's Online Textbook of Bacteriology

The Mechanisms of Bacterial Pathogenicity

Introduction

A **pathogen** is a microorganism that is able to cause disease in a plant, animal or insect. **Pathogenicity** is the ability to produce disease in a host organism. Microbes express their pathogenicity by means of their **virulence**, a term which refers to the **degree of pathogenicity** of the microbe. Hence the **determinants of virulence** of a pathogen are any of its **genetic** or **biochemical** or **structural** features that enable it to produce disease. in a host.

The relationship between a host and a pathogen is dynamic, since each modifies the activities and functions of the other. The outcome of an infection depends on the **virulence of the pathogen** and the relative degree of resistance or susceptibility of the host, due mainly to the effectiveness of the **host defense mechanisms**.

The Underlying Mechanisms of Bacterial Pathogenicity

Two broad qualities of pathogenic bacteria underlie the means by which they cause disease:

1. The ability to invade tissues: Invasiveness, which encompasses mechanisms for **colonization** (adherence and initial multiplication), **ability to bypass or overcome host defense mechanisms**, and **the production of extracellular substances which facilitate invasion**.

2. The ability to produce toxins: Toxigenesis. Bacteria produce two types of toxins called **exotoxins** and **endotoxins**. **Exotoxins** are released from bacterial cells and may act at tissue sites removed from the site of bacterial growth. **Endotoxins** are cell-associated substances that are structural components of the cell walls of Gram-negative bacteria. However, endotoxins may be released from growing bacterial cells or from cells which are lysed as a result of effective host defense (e.g. lysozyme) or the activities of certain antibiotics (e.g. penicillins and cephalosporins). Hence, bacterial toxins, both soluble and cell-associated, may be transported by blood and lymph and cause cytotoxic effects at tissue sites remote

from the original point of invasion or growth. Some bacterial toxins may also act at the site of colonization and play a role in invasion.

COLONIZATION

The first stage of microbial infection is **colonization**: the establishment of the pathogen at the appropriate portal of entry. Pathogens usually colonize host tissues that are in contact with the external environment. Sites of entry in human hosts include the urogenital tract, the digestive tract, the respiratory tract and the conjunctiva. Organisms that infect these regions have usually developed tissue adherence mechanisms and some ability to overcome or withstand the constant pressure of the host defenses on the surface.

Bacterial Adherence to Mucosal Surfaces. In its simplest form, bacterial adherence or attachment to a eukaryotic cell or tissue surface requires the participation of two factors: a **receptor** and an **adhesin**. The receptors so far defined are usually specific carbohydrate or peptide residues on the eukaryotic cell surface. The bacterial adhesin is typically a macromolecular component of the bacterial cell surface which interacts with the host cell receptor. Adhesins and receptors usually interact in a complementary and specific fashion. Table 1 is a list of terms that are used in medical microbiology to refer tomicrobial adherence to surfaces or tissues.

TABLE 1. TERMS USED TO DESCRIBE ADHERENCE FACTORS IN HOST-PARASITE INTERACTIONS

ADHERENCE FACTOR	DESCRIPTION
Adhesin	A surface structure or macromolecule that binds a bacterium to a specific surface
Receptor	A complementary macromolecular binding site on a (eukaryotic) surface that binds specific adhesins or ligands
Lectin	Any protein that binds to a carbohydrate
Ligand	A surface molecule that exhibits specific binding to a receptor molecule on another surface
Mucous	The mucopolysaccharide layer of glucosaminoglycans covering animal cell mucosal surfaces
Fimbriae	Filamentous proteins on the surface of bacterial cells that may behave as adhesins for specific adherence
Common pili	Same as fimbriae
Sex pilus	A specialized pilus that binds mating procaryotes together for the purpose of DNA transfer

Type 1 fimbriae	Fimbriae in *Enterobacteriaceae* which bind specifically to mannose terminated glycoproteins on eukaryotic cell surfaces
Glycocalyx	A layer of exopolysaccharide fibers on the surface of bacterial cells which may be involved in adherence to a surface
Capsule	A detectable layer of polysaccharide (rarely polypeptide) on the surface of a bacterial cell which may mediate specific or nonspecific attachment
Lipopolysaccharide (LPS)	A distinct cell wall component of the outer membrane of Gram-negative bacteria with the potential structural diversity to mediate specific adherence. Probably functions as an adhesin
Teichoic acids and lipoteichoic acids (LTA)	Cell wall components of Gram-positive bacteria that may be involved in nonspecific or specific adherence

Specific Adherence of Bacteria to Cell and Tissue Surfaces

Several types of observations provide indirect evidence for **specificity of adherence** of bacteria to host cells or tissues:

1. **Tissue tropism**: particular bacteria are known to have an apparent preference for certain tissues over others, e.g. *S. mutans* is abundant in dental plaque but does not occur on epithelial surfaces of the tongue; the reverse is true for *S. salivarius* which is attached in high numbers to epithelial cells of the tongue but is absent in dental plaque.

2. **Species specificity**: certain pathogenic bacteria infect only certain species of animals, e.g. *N. gonorrhoeae* infections are limited to humans; Enteropathogenic *E. coli* K-88 infections are limited to pigs; *E. coli* CFA I and CFA II infect humans; *E.coli* K-99 strain infects calves.; Group A streptococcal infections occur only in humans.

3. **Genetic specificity within a species**: certain strains or races within a species are genetically immune to a pathogen , e.g. Certain pigs are not susceptible to *E. coli* K-88 infections; Susceptibility to *Plasmodium vivax* infection (malaria) is dependent on the presence of the Duffy antigens on the host's redblood cells.

Although other explanations are possible, the above observations might be explained by the existence of specific interactions between microorganisms and eukaryotic tissue surfaces which allow microorganisms to become established on the surface.

Mechanisms of Adherence to Cell or Tissue Surfaces

The mechanisms for adherence may involve two steps:

1. **nonspecific adherence: reversible attachment** of the bacterium to the eukaryotic surface (sometimes called "docking")

2. **specific adherence: reversible permanent attachment** of the microorganism to the surface (sometimes called "anchoring").

The usual situation is that reversible attachment precedes irreversible attachment but in some cases, the opposite situation occurs or specific adherence may never occur

Nonspecific adherence involves nonspecific attractive forces which allow approach of the bacterium to the eukaryotic cell surface. Possible interactions and forces involved are:

1. hydrophobic interactions

2. electrostatic attractions

3. atomic and molecular vibrations resulting from fluctuating dipoles of similar frequencies

4. Brownian movement

5. recruitment and trapping by biofilm polymers interacting with the bacterial glycocalyx (capsule)

Specific adherence involves permanent formation of many specific lock-and-key bonds between complementary molecules on each cell surface. Complementary receptor and adhesin molecules must be accessible and arranged in such a way that many bonds form over the area of contact between the two cells. Once the bonds are formed, attachment under physiological conditions becomes virtually irreversible.

Several types of experiments provide **direct evidence that receptor and/or adhesin molecules mediate specificity** of adherence of bacteria to host cells or tissues. These include:

1. The bacteria will bind isolated receptors or receptor analogs.

2. The isolated adhesins or adhesin analogs will bind to the eukaryotic cell surface.

3. Adhesion (of the bacterium to the eukaryotic cell surface) is inhibited by:

a. isolated adhesin or receptor molecules

b. adhesin or receptor analogs

c. enzymes and chemicals that specifically destroy adhesins or receptors

d. antibodies specific to surface components (i.e., adhesins or receptors)

Some Specific Bacterial Adhesins and their Receptors

The adhesins of ***E. coli*** are their common pili or fimbriae. A single strain of *E. coli* is known to be able to express several distinct types of fimbriae encoded by distinct regions of the chromosome or plasmids. This genetic diversity permits an organism to adapt to its changing environment and exploit new opportunities presented by different host surfaces. Many of the adhesive fimbriae of *E. coli* have probably evolved from fimbrial ancestors resembling Type-1 and type 4 fimbriae.

Type-1 fimbriae enable *E. coli* to bind to D-mannose residues on eukaryotic cell surfaces. Type-1 fimbriae are said to be "mannose-sensitive" since exogenous mannose blocks binding to receptors on red blood cells. Although the primary 17kDa fimbrial subunit is the major protein component of Type-1 fimbriae, the mannose-binding site is not located here, but resides in a minor protein (28-31kDa) located at the tips or inserted along the length of the fimbriae. By genetically varying the minor "tip protein" adhesin, the organisms can gain ability to adhere to different receptors. For example, tip proteins on pyelonephritis-associated (pap) pili recognize a galactose-galactose disaccharide, while tip proteins on S-fimbriae recognize sialic acid.

Pseudomonas, Vibrio and ***Neisseria*** possess a fimbrial protein subunit which contains methylated phenylalanine at its amino terminus. These "N-methylphenylalanine pili" have been established as virulence determinants in pathogenesis of *Pseudomonas aeruginosa* lung infection in cystic fibrosis patients. These type of fimbriae occur in *Neisseria gonorrhoeae* and their receptor is thought to be an oligosaccharide.

The adhesins of ***Streptococcus pyogenes***are controversial. In 1972, Gibbons and his colleagues demonstrated that attachment of streptococci to the oral mucosa of mice is dependent on M protein. Olfek and Beachey argued that lipoteichoic acid (LTA), rather than M protein, was responsible for streptococcal adherence to buccal epithelial cells. In 1996, Hasty and Courtney proposed a two-step model of attachment that involved both M protein and teichoic acids. They suggested that LTA loosely tethers streptococci to epithelial cells, and then M protein secures a firmer, irreversible association. In 1992, protein F was
discovered and found to be a fibronectin binding protein. More recently, in 1998, M proteins M1 and M3 were also found to bind to fibronectin. Apparently, *S. pyogenes* produces multiple adhesins with varied specificities.

Staphylococcus aureus also binds to the amino terminus of fibronectin by means of a fibronectin-binding protein which occurs on the bacterial surface. Apparently *S. aureus* and Group A streptococci use different mechanisms but adhere to the same receptor on epithelial surfaces.

Treponema pallidum has three related surface adhesins (P1, P2 and P3) which bind to a four-amino acid sequence (Arg-Gly-Asp-Ser) of the cell-binding domain of fibronectin. It is not clear if T. pallidum uses fibronectin to attach to host surfaces or coats itself with fibronectin to avoid host defenses (phagocytes and immune responses).

TABLE 2. EXAMPLES OF SPECIFIC ATTACHMENTS OF BACTERIA TO HOST CELL OR TISSUE SURFACES

Bacterium	Adhesin	Receptor	Attachment site	Disease
Streptococcus pyogenes	Protein F	Amino terminus of fibronectin	Pharyngeal epithelium	Sore throat
Streptococcus mutans	Glycosyl transferase	Salivary glycoprotein	Pellicle of tooth	Dental caries
Streptococcus salivarius	Lipoteichoic acid	Unknown	Buccal epithelium of tongue	None
Streptococcus pneumoniae	Cell-bound protein	N-acetylhexosamine-galactose disaccharide	Mucosal epithelium	pneumonia
Staphylococcus aureus	Cell-bound protein	Amino terminus of fibronectin	Mucosal epithelium	Various
Neisseria gonorrhoeae	N-methylphenyl-alanine pili	Glucosamine-galactose carbohydrate	Urethral/ cervical epithelium	Gonorrhea
Enterotoxigenic E. coli	Type-1 fimbriae	Species-specific carbohydrate(s)	Intestinal epithelium	Diarrhea
Uropathogenic *E. coli*	Type 1 fimbriae	Complex carbohydrate	Urethral epithelium	Urethritis
Uropathogenic *E. coli*	P-pili (pap)	Globobiose linked to ceramide lipid	Upper urinary tract	Pyelonephritis

Bordetella pertussis	Fimbriae ("filamentous hemagglutinin")	Galactose on sulfated glycolipids	Respiratory epithelium	Whooping cough
Vibrio cholerae	N-methylphenylalanine pili	Fucose and mannose carbohydrate	Intestinal epithelium	Cholera
Treponema pallidum	Peptide in outer membrane	Surface protein (fibronectin)	Mucosal epithelium	Syphilis
Mycoplasma	Membrane protein	Sialic acid	Respiratory epithelium	Pneumonia
Chlamydia	Unknown	Sialic acid	Conjunctival or urethral epithelium	Conjunctivitis or urethritis

INVASION

The invasion of a host by a pathogen may be aided by the production of bacterial extracellular substances which act against the host by breaking down primary or secondary defenses of the body. Medical microbiologists have long referred to these substances as **invasins**. Invasins are proteins (enzymes) that act locally to damage host cells and/or have the immediate effect of facilitating the growth and spread of the pathogen. The damage to the host as a result of this invasive activity may become part of the pathology of an infectious disease.

The extracellular proteins produced by bacteria which promote their invasion are not clearly distinguished from some extracellular protein toxins ("exotoxins") which also damage the host. Invasins usually act at a short range (in the immediate vicinity of bacterial growth) and may not actually kill cells in their range of activity; exotoxins are often cytotoxic and may act at remote sites (removed from the site of bacterial growth). Also, exotoxins typically are more specific and more potent in their activity than invasins. Even so, some classic exotoxins (e.g. diphtheria toxin, anthrax toxin) may play some role in invasion in the early stages of an infection, and some invasins (e.g. staphylococcal leukocidin) have a relatively specific cytopathic effect.

A Survey of Bacterial Invasins

Spreading Factors

"Spreading Factors" is a descriptive term for a family of bacterial enzymes that affect the physical properties of tissue matrices and intercellular spaces, thereby promoting the spread of the pathogen.

Hyaluronidase. is the original spreading factor It is produced by streptococci. staphylococci, and

clostridia. The enzyme attacks the interstitial cement ("ground substance") of connective tissue by depolymerizing hyaluronic acid.

Collagenase is produced by *Clostridium histolyticum* and *Clostridium perfringens*. It breaks down collagen, the framework of muscles, which facilitates gas gangrene due to these organisms.

Neuraminidase is produced by intestinal pathogens such as *Vibrio cholerae* and *Shigella dysenteriae*. It degrades neuraminic acid (also called sialic acid), an intercellular cement of the epithelial cells of the intestinal mucosa.

Streptokinase and **Staphylokinase** are produced by streptococci and staphylococci, respectively. Kinase enzymes convert inactive plasminogen to plasmin which digests fibrin and prevents clotting of the blood. The relative absence of fibrin in spreading bacterial lesions allows more rapid diffusion of the infectious bacteria.

Enzymes that Cause Hemolysis and/or Leucolysis

These enzymes usually act on the animal cell membrane by insertion into the membrane (forming a pore that results in cell lysis), or by enzymatic attack on phospholipids, which destabilizes the membrane. They may be referred to as **lecithinases** or **phospholipases**, and if they lyse red blood cells they are sometimes called **hemolysins**. **Leukocidins**, produced by staphylococci and **streptolysin** produced by **streptococci** specifically lyse phagocytes and their granules. These latter two enzymes are also considered to be bacterial exotoxins.

Phospholipases, produced by *Clostridium perfringens* (i.e., alpha toxin), hydrolyze phospholipids in cell membranes by removal of polar head groups.

Lecithinases, also produced by *Clostridium perfringens*, destroy lecithin (phosphatidylcholine) in cell membranes.

Hemolysins, notably produced by staphylococci (i.e., alpha toxin), streptococci (i.e.,streptolysin) and various clostridia, may be channel-forming proteins or phospholipases or lecithinases that destroy red blood cells and other cells (i.e., phagocytes) by lysis.

Staphylococcal coagulase

Coagulase, formed by *Staphylococcus aureus*, is a cell-associated and diffusible enzyme that converts fibrinogen to fibrin which causes clotting. Coagulase activity is almost alway associated with pathogenic *S. aureus* and almost never associated with nonpathogenic *S. epidermidis*, which has led to much speculation as to its role as a determinant of virulence. Possibly, cell bound coagulase could provide an antigenic disguise if it clotted fibrin on the cell surface. Or a staphylococcal lesion encased in fibrin (e.g. a boil or pimple) could make the bacterial cells resistant to phagocytes or tissue bactericides or even

drugs which might be unable to diffuse to their bacterial target.

Extracellular Digestive Enzymes

Heterotrophic bacteria, in general, produce a wide variety of extracellular enzymes including **proteases**, **lipases**, **glycohydrolases**, **nucleases**, etc., which are not clearly shown to have a direct role in invasion or pathogenesis. These enzymes presumably have other functions related to bacterial nutrition or metabolism, but may aid in invasion either directly or indirectly.

Toxins With Short-Range Effects Related to Invasion

Bacterial protein toxins which have adenylate cyclase activity, are thought to have immediate effects on host cells that promote bacterial invasion. One component of the anthrax toxin (**EF** or **Edema Factor**) is an **adenylate cyclase** that acts on nearby cells to cause increased levels of cyclic AMP and disruption of cell permeability. One of the toxins of *Bordetella pertussis*, the agent of whooping cough, has a similar effect. These toxins may contribute to invasion through their effetcts on macrophages or lymphocytes in the vicinity which are playing an essential role to contain the infection.

The following table summarizes the activities of many bacterial proteins that are noted for their contribution to bacterial invasion of tissues.

TABLE 3. SOME EXTRACELLULAR BACTERIAL PROTEINS THAT ARE CONSIDERED INVASINS

Invasin	Bacteria Involved	Activity
Hyaluronidase	Streptococci, staphylococci and clostridia	Degrades hyaluronic of connective tissue
Collagenase	*Clostridium* species	Dissolves collagen framework of muscles
Neuraminidase	*Vibrio cholerae* and *Shigella dysenteriae*	Degrades neuraminic acid of intestinal mucosa
Coagulase	*Staphylococcus aureus*	Converts fibrinogen to fibrin which causes clotting
Kinases	Staphylococci and streptococci	Converts plasminogen to plasmin which digests fibrin
Leukocidin	*Staphylococcus aureus*	Disrupts neutrophil membranes and causes discharge of lysosomal granules
Streptolysin	*Streptococcus pyogenes*	Repels phagocytes and disrupts phagocyte membrane and causes discharge of lysosomal granules

Hemolysins	Streptococci, staphylococci and clostridia	Phospholipases or lecithinases that destroy red blood cells (and other cells) by lysis
Lecithinases	*Clostridium perfringens*	Destroy lecithin in cell membranes
Phospholipases	*Clostridium perfringens*	Destroy phospholipids in cell membrane
Anthrax EF	*Bacillus anthracis*	One component (EF) is an adenylate cyclase which causes increased levels of intracellular cyclic AMP
Pertussis AC	*Bordetella pertussis*	One toxin component is an adenylate cyclase that acts locally producing an increase in intracellular cyclic AMP

EVASION OF HOST DEFENSES

Some pathogenic bacteria are inherently able to resist the bactericidal components of host tissues. For example, the poly-D-glutamate capsule of *Bacillus anthracis* protects the organisms against cell lysis by cationic proteins in sera or in phagocytes. The outer membrane of Gram-negative bacteria is a formidable permeability barrier that is not easily penetrated by hydrophobic compounds such as bile salts which are harmful to the bacteria. Pathogenic mycobacteria have a waxy cell wall that resists attack or digestion by most tissue bactericides. And intact lipopolysaccharides (LPS) of Gram-negative pathogens may protect the cells from complement-mediated lysis or the action of lysozyme.

Most successful pathogens, however, possess additional structural or biochemical features which allow them to resist the main lines of host internal defense against them, i.e., the phagocytic and immune responses of the host.

Overcoming Host Phagocytic Defenses

Microorganisms invading tissues are first and foremost exposed to phagocytes. Bacteria that readily attract phagocytes, and that are easily ingested and killed, are generally unsuccessful as parasites. In contrast, most bacteria that are successful as parasites interfere to some extent with the activities of phagocytes or in some way avoid their attention.

Microbial strategies to avoid phagocytic killing are numerous and diverse, but are usually aimed at blocking one or of more steps in the phagocytic process. Recall the steps in phagocytosis:

1. Contact between phagocyte and microbial cell

2. Engulfment

3. Phagosome formation

4. Phagosome-lysosome fusion

5. Killing and digestion

Avoiding Contact with Phagocytes

Bacteria can avoid the attention of phagocytes in a number of ways.

1. Invade or remain confined in regions inaccessible to phagocytes. Certain internal tissues (e.g. the lumen of glands) and surface tissues (e.g. the skin) are not patrolled by phagocytes.

2. Avoid provoking an overwhelming inflammatory response. Some pathogens induce minimal or no inflammation required to focus the phagocytic defenses.

3. Inhibit phagocyte chemotaxis. e.g. Streptococcal streptolysin (which also kills phagocytes) suppresses neutrophil chemotaxis, even in very low concentrations. Fractions of *Mycobacterium tuberculosis* are known to inhibit leukocyte migration. *Clostridium ø* toxin inhibits neutrophil chemotaxis.

4. Hide the antigenic surface of the bacterial cell. Some pathogens can cover the surface of the bacterial cell with a component which is seen as "self" by the host phagocytes and immune system. Phagocytes cannot recognize bacteria upon contact and the possibility of opsonization by antibodies to enhance phagocytosis is minimized. For example, pathogenic *Staphylococcus aureus* produces cell-bound coagulase which clots fibrin on the bacterial surface. *Treponema pallidum* binds fibronectin to its surface. Group A streptococci are able to synthesize a capsule composed of hyaluronic acid.

Inhibition of Phagocytic Engulfment

Some bacteria employ strategies to **avoid engulfment (ingestion)** if phagocytes do make contact with them. Many important pathogenic bacteria bear on their surfaces substances that inhibit phagocytic adsorption or engulfment. Clearly it is the bacterial surface that matters. Resistance to phagocytic ingestion is usually due to a component of the bacterial cell wall, or fimbriae, or a capsule enclosing the bacterial wall. Classical examples of antiphagocytic substances on the bacterial surface include:

Polysaccharide capsules of *S. pneumoniae*, *Haemophilus influenzae*, *Treponema pallidum* and *Klebsiella pneumoniae*

M protein and fimbriae of Group A streptococci

Surface slime (polysaccharide) produced by *Pseudomonas aeruginosa*

O antigen associated with LPS of *E. coli*

K antigen of *E. coli* or the analogous Vi antigen of *Salmonella typhi*

Cell-bound or soluble Protein A produced by *Staphylococcus aureus*

Survival Inside of Phagocytes

Some bacteria survive inside of phagocytic cells, in either neutrophils or macrophages. Bacteria that can resist killing and survive or multiply inside of phagocytes are considered intracellular parasites. The environment of the phagocyte may be a protective one, protecting the bacteria during the early stages of infection or until they develop a full complement of virulence factors. The intracellular environment guards the bacteria against the activities of extracellular bactericides, antibodies, drugs, etc.

Most intracellular parasites have special (genetically-encoded) mechanisms to get themselves into their host cell as well as special mechanisms to survive once they are inside. Intracellular parasites usually survive by virtue of mechanisms which interfere with the bactericidal activities of the host cell. Some of these bacterial mechanisms include:

1. **Inhibition of phagosome-lysosome fusion**. The bacteria survive inside of phagosomes because they prevent the discharge of lysosomal contents into the phagosome environment. Specifically phagolysosome formation is inhibited in the phagocyte. This is the strategy employed by *Salmonella, M. tuberculosis, Legionella* and the *Chlamydiae*.

2. **Survival inside the phagolysosome**. With some intracellular parasites, phagosome-lysosome fusion occurs but the bacteria are resistant to inhibition and killing by the lysosomal constituents. Also, some extracellular pathogens can resist killing in phagocytes utilizing similar resistance mechanisms. Little is known of how bacteria can resist phagocytic killing within the phagocytic vacuole, but it may be due to the surface components of the bacteria or due to extracellular substances that they produce which interfere with the mechanisms of phagocytic killing. *Bacillus anthracis*, *Mycobacterium tuberculosis* and *Staphylococcus aureus* all possess mechanisms to survive intracellular killing in macrophages.

3. **Escape from the phagosome**. Early escape from the phagosome vacuole is essential for growth and virulence of some intracellular pathogens. This is a very clever strategy employed by the Rickettsias which produce a phospholipase enzyme that lyses the phagosome membrane within thirty secondes of after ingestion.

Products of Bacteria that Kill or Damage Phagocytes

One obvious strategy in defense against phagocytosis is direct attack by the bacteria upon the professional phagocytes. Any of the substances that pathogens produce that cause damage to phagocytes have been referred to as "aggressins". Most of these are actually extracellular enzymes or toxins that kill phagocytes. Phagocytes may be killed by a pathogen before or after ingestion.

Killing phagocytes before ingestion. Many Gram-positive pathogens, particularly the pyogenic cocci, secrete extracellular enzymes which kill phagocytes. Many of these enzymes are called "hemolysins" because their activity in the presence of red blood cells results in the lysis of the rbcs.

Pathogenic streptococci produce streptolysin. Streptolysin O binds to cholesterol in membranes. The effect on neutrophils is to cause lysosomal granules to explode, releasing their contents into the cell cytoplasm.

Pathogenic staphylococci produce leukocidin, which also acts on the neutrophil membrane and causes discharge of lysosomal granules.

Other examples of bacterial extracellular proteins that inhibit phagocytosis include the Exotoxin A of *Pseudomonas aeruginosa* which kills macrophages, and the bacterial exotoxins that are adenylate cyclases (e.g. anthrax toxin EF and pertussis AC) which decrease phagocytic activity.

Killing phagocytes after ingestion. Some bacteria exert their toxic action on the phagocyte after ingestion has taken place. They may grow in the phagosome and release substances which can pass through the phagosome membrane and cause discharge of lysosomal granules, or they may grow in the phagolysosome and release toxic substances which pass through the phagolysosome membrane to other target sites in the cell. Many bacteria which are the intracellular parasites of macrophages (e.g Mycobacteria, *Brucella*, *Listeria*) usually destroy macrophages in the end, but the mechanisms are not understood.

Overcoming Host Phagocytic Defenses

On epithelial surfaces the main antibacterial immune defense of the host is the protection afforded by secretory antibody (IgA). Once the epithelial surfaces have been penetrated, however, the major host defenses of inflammation, complement, phagocytosis, Antibody-mediated Immunity (AMI), and Cell-mediated Immunity (CMI) are encountered. If there is a way for a pathogen to successfully bypass or overcome these host defenses, then some bacterial pathogen has probably discovered it. Bacteria evolve very rapidly in relation to their host, so that most of the feasible anti-host strategies are likely to have been tried out and exploited. Ability to defeat the immune defenses may play a major role in the virulence of a bacterium and in the pathology of disease. Several strategic bacterial defenses are described below.

Immunological Tolerance to a Bacterial Antigen

Tolerance is a property of the host in which there is an immunologically-specific reduction in the immune response to a given Ag. Tolerance to a bacterial Ag does not involve a general failure in the immune response but a particular deficiency in relation to the specific antigen(s) of a given bacterium. If there is a depressed immune response to relevant antigens of a parasite, the process of infection is

facilitated. Tolerance can involve either AMI or CMI or both arms of the immunological response.

Tolerance to an Ag can arise in a number of ways, but three are possibly relevant to bacterial infections.

1. **Fetal exposure to Ag**

2. **High persistent doses of circulating Ag**

3. **Molecular mimicry**. If a bacterial Ag is very similar to normal host "antigens", the immune responses to this Ag may be weak giving a degree of tolerance. Resemblance between bacterial Ag and host Ag is referred to as molecular mimicry. In this case the antigenic determinants of the bacterium are so closely related chemically to host "self" components that the immunological cells cannot distinguish between the two and an immune response cannot be raised. Some bacterial capsules are composed of polysaccharides (hyaluronic acid, sialic acid) so similar to host tissue polysaccharides that they are not immunogenic.

Antigenic Disguise

Bacteria may be able to coat themselves with host proteins (fibrin, fibronectin, antibody molecules) or with host polysaccharides (sialic acid, hyaluronic acid) so that they are able to hide their own antigenic surface components from the immunological system.

Immunosuppression

Some pathogens (mainly viruses and protozoa, rarely bacteria) cause immunosuppression in the infected host. This means that the host shows depressed immune responses to antigens in general, including those of the infecting pathogen. Suppressed immune responses are occasionally observed during chronic bacterial infections such as leprosy and tuberculosis.

Persistence of a Pathogen at Bodily Sites Inaccessible to the Immune Response

Some pathogens can avoid exposing themselves to immune forces.

Intracellular pathogens can evade host immune responses as long as they stay inside of infected cells and they do not allow microbial Ag to form on the cell surface. Macrophages support the growth of the bacteria and at the same time give them protection from immune responses.

Some pathogens persist on the luminal surfaces of the GI tract, oral cavity and the urinary tract, or the lumen of the salivary gland, mammary gland or the kidney tubule.

Induction of Ineffective Antibody

Many types of antibody are formed against a given Ag, and some bacterial components may display various antigenic determinants. Antibodies tend to range in their capacity to react with Ag (the ability of specific Ab to bind to an Ag is called **avidity**). If Abs formed against a bacterial Ag are of low avidity, or if they are directed against unimportant antigenic determinants, they may have only weak antibacterial action. Such "ineffective" (non-neutralizing) Abs might even aid a pathogen by combining with a surface Ag and blocking the attachment of any functional Abs that might be present.

Antibodies Absorbed by Soluble Bacterial Antigens

Some bacteria can liberate antigenic surface components in a soluble form into the tissue fluids. These soluble antigens are able to combine with and "neutralize" antibodies before they reach the bacterial cells. For example, small amounts of endotoxin (LPS) may be released into surrounding fluids by Gram-negative bacteria.

Antigenic Variation

One way bacteria can avoid forces of the immune response is by periodically changing antigens, i.e., undergoing antigenic variation. Some bacteria avoid the host antibody response by changing from one type of fimbriae to another, by switching fimbrial tips. This makes the original AMI response obsolete by using new fimbriae that do not bind the previous antibodies. Pathogenic bacteria can vary (change) other surface proteins that are the targets of antibodies. Antigenic variation is prevalent among pathogenic viruses as well.

Changing antigens during the course of an infection

Antigens may vary or change within the host during the course of an infection, or alternatively antigens may vary among multiple strains (antigenic types) of a parasite in the population. Antigenic variation is an important mechanism used by pathogenic microorganisms for escaping the neutralizing activities of antibodies. Antigenic variation usually results from site-specific inversions or gene conversions or gene rearrangements in the DNA of the microorganisms.

Changing antigens between infections

Many pathogenic bacteria exist in nature as multiple antigenic types or serotypes, meaning that they are variant strains of the same pathogenic species. For example, there are multiple serotypes of *Salmonella typhimurium* based on differences in cell wall (O) antigens or flagellar (H) antigens. There are 80 different antigenic types of *Streptococcus pyogenes* based on M-proteins on the cell surface. There are over one hundred strains of *Streptococcus pneumoniae* depending on their capsular polysaccharide antigens. Based on minor differences in surface structure chemistry there are multiple serotypes of *Vibrio cholerae*, *Staphylococcus aureus*, *Escherichia coli*, *Neisseria gonorrhoeae* and an assortment of other bacterial pathogens.

TOXIGENESIS

Two types of bacterial toxins

At a chemical level there are two types of bacterial toxins:

lipopolysaccharides, which are associated with the cell walls of Gram-negative bacteria.

proteins, which may be released into the extracellular environment of pathogenic bacteria.

The lipopolysaccharide (LPS) component of the Gram-negative bacterial outer membrane bears the name endotoxin because of its association with the cell wall of bacteria.

Most of the protein toxins are thought of as exotoxins, since they are "released" from the bacteria and act on host cells at a distance.

BACTERIAL PROTEIN TOXINS

The protein toxins are typically soluble proteins secreted by living bacteria during exponential growth. The production of protein toxins is generally specific to a particular bacterial species (e.g. only *Clostridium tetani* produces tetanus toxin; only *Corynebacterium diphtheriae* produces the diphtheria toxin). Usually, virulent strains of the bacterium produce the toxin (or range of toxins) while nonvirulent strains do not, such that the toxin is the major determinant of virulence. Both Gram-positive and Gram-negative bacteria produce soluble protein toxins. Bacterial protein toxins are the most potent poisons known and may show activity at very high dilutions.

The protein **toxins resemble enzymes** in a number of ways. Like enzymes, bacterial exotoxins:

are **proteins**

are **denatured by heat**, acid, proteolytic enzymes

have a **high biological activity** (most act catalytically)

exhibit **specificity** of action

As enzymes attack specific substrates, so bacterial protein toxins are **highly specific** in the substrate utilized and in their mode of action. The substrate (in the host) may be a component of tissue cells, organs, or body fluid. Usually the site of damage caused by the toxin indicates the location of the substrate for that toxin. Terms such as "enterotoxin", "neurotoxin", "leukocidin" or "hemolysin" are sometimes used to indicate the target site of some well-defined protein toxins.

Certain protein toxins have very specific **cytotoxic activity** (i.e., they attack specific cells, for example, tetanus or botulinum toxins), but some (as produced by staphylococci, streptococci, clostridia, etc.) have fairly broad cytotoxic activity and cause nonspecific death of tissues (necrosis). Toxins that are phospholipases may be relatively nonspecific in their cytotoxicity because they cleave phospholipids which are components of host cell membranes resulting in the death of the cell by leakage of cellular contents. This is also true of pore-forming "hemolysins" and "leukocidins".

A few protein toxins obviously bring about the death of the host and are known as "lethal toxins", and even though the tissues affected and the target sites may be known, the precise mechanism by which death occurs is not understood (e.g. anthrax toxin).

As "foreign" substances to the host, most of the protein toxins are **strongly antigenic**. In vivo, **specific antibody (antitoxin) neutralizes the toxicity** of these bacterial proteins. However, in vitro, specific antitoxin may not fully inhibit their enzymatic activity. This suggests that the antigenic determinant of the toxin is distinct from the active (enzymatic) portion of the protein molecule. The degree of neutralization of the enzymatic site may depend on the distance from the antigenic site on the molecule. However, since the toxin is fully neutralized in vivo, this suggests that other (host) factors must play a role.

Protein toxins are inherently unstable: in time they lose their toxic properties but retain their antigenic ones. This was first discovered by Ehrlich and he coined the term toxoid for this product. **Toxoids** are detoxified toxins which retain their antigenicity and their immunizing capacity. The formation of toxoids can be accelerated by treating toxins with a variety of reagents including formalin, iodine, pepsin, ascorbic acid, ketones, etc. The mixture is maintained at 37º at pH range 6 to 9 for several weeks. The resulting toxoids can be use for artificial immunization against diseases caused by pathogens where the primary determinant of bacterial virulence is toxin production. Toxoids are the immunizing agents against diphtheria and tetanus that are part of the DPT vaccine.

A + B Subunit Arrangement of Protein Toxins

Many protein toxins, notably those that act intracellularly (with regard to host cells), consist of two components: one component (subunit A) is responsible for the enzymatic activity of the toxin; the other component (subunit B) is concerned with binding to a specific receptor on the host cell membrane and transferring the enzyme across the membrane. The enzymatic component is not active until it is released from the native toxin. Isolated A subunits are enzymatically active and but lack binding and cell entry capability. Isolated B subunits may bind to target cells (and even block the binding of the native A+B toxin), but they are nontoxic. There are a variety of ways that toxin subunits may be synthesized and arranged: **A-B** or **A-5B** indicates that subunits synthesized separately and associated by noncovalent bonds; **A/B** denotes subunit domains of a single protein that may be separated by proteolytic cleavage; **A + B** indicates separate protein subunits that interact at the target cell surface; **5B** indicates that the binding domain is composed of 5 identical subunits.

Attachment and Entry of Toxins

There are at least two mechanisms of toxin entry into target cells. In one mechanism called **direct entry**, the B subunit of the native toxin (A+B) binds to a specific receptor on the target cell and induces the formation of a pore in the membrane through which the A subunit is transferred into the cell cytoplasm. In an alternative mechanism, the native toxin binds to the target cell and the A+B structure is taken into the cell by the process of **receptor-mediated endocytosis** (**RME**). The toxin is internalized in the cell in a membrane-enclosed vesicle called an endosome. H^+ ions enter the endosome lowering the internal pH which causes the A+B subunits to separate. Somehow, the B subunit affects the release of the A subunit from the endosome so that it will reach its target in the cell cytoplasm. The B subunit remains in the endosome and is recycled to the cell surface. In both cases, a large protein molecule must insert into and cross a membrane lipid bilayer. This activity is reflected in the ability of most A/B native toxins, or their B components, to insert into artificial lipid bilayers, creating ion permeable pathways.

Other Considerations

In keeping with the observation that genetic information for functions not involved in viability of bacteria is frequently located extrachromosomally, the genes encoding toxin production are generally located on plasmids or in lysogenic bacteriophages. Thus the processes of genetic exchange in bacteria, notably conjugation and transduction, can mobilize these genetic elements between strains of bacteria, and therefore may play a role in determining the pathogenic potential of a bacterium.

Why certain bacteria produce such potent toxins is mysterious and is analogous to asking why an organism should produce an antibiotic. The production of a toxin may play a role in adapting a bacterium to a particular niche, but it is not essential to the viability of the organism. Many toxigenic bacteria are free-living in Nature and in associations with humans in a form which is phenotypically identical to the toxigenic strain but lacking the ability to produce the toxin.

There is conclusive evidence for the pathogenic role of diphtheria, tetanus and botulinum toxins, various enterotoxins, staphylococcal toxic shock syndrome toxin, and streptococcal erythrogenic toxin. And there is clear evidence for the pathological involvement of pertussis toxin, anthrax toxin, shiga toxin and the necrotizing toxins of clostridia in host-parasite relationships.

Table 4. SOURCES AND ACTIVITIES OF BACTERIAL TOXINS

NAME OF TOXIN	BACTERIUM INVOLVED	ACTIVITY

Anthrax toxin (EF)	*Bacillus anthracis*	Edema Factor (EF) is an adenylate cyclase that causes increased levels in intracellular cyclic AMP in phagocytes and formation of ion-permeable pores in membranes (hemolysis)
Adenylate cyclase toxin	*Bordetella pertussis*	Acts locally to increase levels of cyclic AMP in phagocytes and formation of ion-permeable pores in membranes (hemolysis)
Cholera enterotoxin	*Vibrio cholerae*	ADP ribosylation of G proteins stimulates adenlyate cyclase and increases cAMP in cells of the GI tract, causing secretion of water and electrolytes
E. coli LT toxin	*Escherichia coli*	Similar to cholera toxin
Shiga toxin	*Shigella dysenteriae*	Enzymatically cleaves rRNA resulting in inhibition of protein synthesis in susceptible cells
Botulinum toxin	*Clostridium botulinum*	Zn^{++} dependent protease that inhibits neurotransmission at neuromuscular synapses resulting in flaccid paralysis
Tetanus toxin	*Clostridium tetani*	Zn^{++} dependent protease that inhibits neurotransmission at inhibitory synapses resulting in spastic paralysis
Diphtheria toxin	*Corynebacterium diphtheriae*	ADP ribosylation of elongation factor 2 leads to inhibition of protein synthesis in target cells
Pertussis toxin	*Bordetella pertussis*	ADP ribosylation of G proteins blocks inhibition of adenylate cyclase in susceptible cells
Staphylococcus enterotoxins*	*Staphylococcus aureus*	Massive activation of the immune system, including lymphocytes and macrophages, leads to emesis (vomiting)
Toxic shock syndrome toxin (TSST-1)*	*Staphylococcus aureus*	Acts on the vascular system causing inflammation, fever and shock
Erythrogenic toxin (scarlet fever toxin)*	*Streptococcus pyogenes*	Causes localized erythematous reactions

*** The "pyrogenic exotoxins" produced by *Staphylococcus aureus* and *Streptococcus pyogenes* have been**

designated as superantigens. They represent a family of molecules with the ability to elicit massive activation of the immune system. These proteins share the ability to stimulate T cell proliferation by interaction with Class II MHC molecules on APCs and specific V beta chains of the T cell receptor. The important feature of this interaction is the resultant production of IL-1, TNF, and other lymphokines which appear to be the principal mediators of disease processes associated with these toxins.

ENDOTOXINS

Endotoxins are part of the outer cell wall of bacteria. Endotoxins are invariably associated with Gram-negative bacteria as constituents of the outer membrane of the cell wall. Although the term **endotoxin** is occasionally used to refer to any "cell-associated" bacterial toxin, it should be reserved for the lipopolysaccharide complex associated with the outer envelope of Gram-negative bacteria such as *E. coli*, *Salmonella*, *Shigella*, *Pseudomonas*, *Neisseria*, *Haemophilus*, and other leading pathogens. Lipopolysaccharide (LPS) participates in a number of outer membrane functions that are essential for bacterial growth and survival, especially within the context of a host-parasite interaction.

The biological activity of endotoxin is associated with the **lipopolysaccharide** (**LPS**). Toxicity is associated with the lipid component (**Lipid A**) and immunogenicity (antigenicity) is associated with the polysaccharide components. The cell wall antigens (**O antigens**) of Gram-negative bacteria are components of LPS. LPS activates complement by the alternative (properdin) pathway and may be a part of the pathology of most Gram-negative bacterial infections.

For the most part, endotoxins remain associated with the cell wall until disintegration of the bacteria. In vivo, this results from autolysis, external lysis, and phagocytic digestion of bacterial cells. It is known, however, that small amounts of endotoxin may be released in a soluble form, especially by young cultures.

Compared to the classic exotoxins of bacteria, endotoxins are less potent and less specific in their action, since they do not act enzymatically. Endotoxins are heat stable (boiling for 30 minutes does not destabilize endotoxin), but certain powerful oxidizing agents such as , superoxide, peroxide and hypochlorite degrade them. Endotoxins, although strongly antigenic, cannot be converted to toxoids. A comparison of the properties of bacterial endotoxins compared to classic exotoxins is shown in Table 5.

Table 5. CHARACTERISTICS OF BACTERIAL ENDOTOXINS AND EXOTOXINS

PROPERTY	ENDOTOXIN	EXOTOXIN
CHEMICAL NATURE	Lipopolysaccharide(mw = 10kDa)	Protein (mw = 50-1000kDa)
RELATIONSHIP TO CELL	Part of outer membrane	Extracellular, diffusible
DENATURED BY BOILING	No	Usually
ANTIGENIC	Yes	Yes

FORM TOXOID	No	Yes
POTENCY	Relatively low (>100ug)	Relatively high (1 ug)
SPECIFICITY	Low degree	High degree
ENZYMATIC ACTIVITY	No	Usually
PYROGENICITY	Yes	Occasionally

Lipopolysaccharides are complex amphiphilic molecules with a mw of about 10kDa, that vary widely in chemical composition both between and among bacterial species. In a basic groundplan common to all endotoxins, LPS consists of three components or regions:

(1) Lipid A---- (2) Core polysaccharide---- (3) O polysaccharide

Lipid A is the lipid component of LPS. It contains the hydrophobic, membrane-anchoring region of LPS. Lipid A consists of a phosphorylated N-acetylglucosamine (NAG) dimer with 6 or 7 fatty acids (FA) attached. Usually 6 FA are found. All FA in Lipid A are saturated. Some FA are attached directly to the NAG dimer and others are esterified to the 3-hydroxy fatty acids that are characteristically present. The structure of Lipid A is highly conserved among Gram-negative bacteria. Among ***Enterobacteriaceae*** Lipid A is virtually constant.

The **Core (R) polysaccharide** is attached to the 6 position of one NAG. The R antigen consists of a short chain of sugars. For example: KDO - Hep - Hep - Glu - Gal - Glu - GluNAc.
Two unusual sugars are usually present, heptose and 2-keto-3-deoxyoctonoic acid (KDO), in the core polysaccharide. KDO is unique and invariably present in LPS and so has been an indicator in assays for LPS (endotoxin).

With minor variations, the core polysaccharide is common to all members of a bacterial genus (e.g. *Salmonella*), but it is structurally distinct in other genera of Gram-negative bacteria. *Salmonella*, *Shigella* and *Escherichia* have similar but not identical cores.

The **O polysaccharide** (also referred to as the **O antigen** or **O side chain**) is attached to the core polysaccharide. It consists of repeating oligosaccharide subunits made up of 3-5 sugars. The individual chains vary in length ranging up to 40 repeat units. The O polysaccharide is much longer than the core polysaccharide and it maintains the hydrophilic domain of the LPS molecule. Often, a unique group of sugars, called **dideoxyhexoses**, occurs in the O polysaccharide.

A major antigenic determinant (antibody-combining site) of the Gram-negative cell wall resides in the O polysaccharide. Great variation occurs in the composition of the sugars in the O side chain between species and even strains of Gram-negative bacteria.

LPS and virulence of Gram-negative bacteria

Endotoxins are toxic to most mammals. They are strong antigens but they seldom elicit immune responses which give full protection to the animal against secondary challenge with the endotoxin. They cannot be toxoided. Endotoxins released from multiplying or disintegrating bacteria significantly contribute to the symptoms of Gram-negative bacteremia and septicemia, and therefore represent important pathogenic factors in Gram-negative infections. Regardless of the bacterial source, all endotoxins produce the same range of biological effects in the animal host. The injection of living or killed Gram-negative cells, or purified LPS, into experimental animals causes a wide spectrum of nonspecific **pathophysiological reactions related to inflammation** such as:

fever

changes in white blood cell counts

disseminated intravascular coagulation

tumor necrosis

hypotension

shock

lethality

The sequence of events follows a regular pattern: 1. latent period; 2. physiological distress (fever, diarrhea, prostration, shock); 3. death. How soon death occurs varies on the dose of the endotoxin, route of administration, and species of animal. Animals vary in their susceptibility to endotoxin.

The role of Lipid A

The physiological activities of endotoxins are mediated mainly by the Lipid A component of LPS. Lipid A is the toxic component of LPS, as evidence by the fact that injection of purified Lipid A into an experimental animal will elicit the same response as intact LPS. The primary structure of Lipid A has been elucidated, and Lipid A has been chemically synthesized. Its biological activity appears to depend on a peculiar conformation that is determined by the glucosamine disaccharide, the PO_4 groups, the acyl chains, and also the KDO-containing inner core. Lipid A is known to react at the surfaces of macrophages causing them to release cytokines that mediate the pathophysiological response to endotoxin.

The role of the O polysaccharide

Although nontoxic, the polysaccharide side chain (O antigen) of LPS may act as a determinant of

virulence in Gram-negative bacteria. The O polysaccharide is responsible for the property of "smoothness" of bacterial cells, which may contribute to their resistance to phagocytic engulfment. The O polysaccharide is hydrophilic and may allow diffusion or delivery of the toxic lipid in the hydrophilic (in vivo) environment. The long side chains of LPS afforded by the O polysaccharide may prevent host complement from depositing on the bacterial cell surface which would bring about bacterial cell lysis. The O polysaccharide may supply a bacterium with its specific ligands (adhesins) for colonization which is essential for expression of virulence. Lastly, the O-polysaccharide is antigenic, and the usual basis for antigenic variation in Gram-negative bacteria rests in differences in their O polysaccharides.

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